IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
HEATH, *et al.*
Serial No.: 08/699,716
Filed: 27 August 1996
For: RECOMBINANT F1-V PLAGUE VACCINE

Art Unit: 1645
Examiner: Duffy, Patricia Ann
Atty. Dckt: 003/029/SAP

AFFIDAVIT OF SUSAN L. WELKOS

1. I, Susan L. Welkos, an inventor of the above-referenced application and resident of Frederick, MD, declare the following:
2. My curriculum vitae is attached.
3. Arthur M. Friedlander, David G. Heath, George W. Anderson, Jr. and I are joint inventors of the subject matter disclosed in the above-referenced application.
4. Exhibit SW1 (DH5) is from my personal notebook which contains my notes on Army Plague Vaccine Group meetings.
5. Exhibit SW2 (DH8) is from my personal notebook which contains my notes on Army Plague Vaccine Group meetings.
6. Exhibit SW3 (AF2) is from my personal notebook which contains my notes on Army Plague Vaccine Group meetings.
7. Exhibit SW4 (AF4) is from my personal notebook which contains my notes on Army Plague Vaccine Group meetings.
8. Exhibit SW5 (DH6B) is from my personal notebook which contains my notes on Army Plague Vaccine Group meetings.
9. The *Yersinia pestis* strains C12 and CO92 for all the challenge studies conducted by George W. Anderson, Jr. were provided by me.
10. From **[redacted date which is before 13 March 1996]** to at least December 1996, I conducted research and development on a plague vaccine comprising a F1-V fusion protein as an immunogen as part of the Army Plague Vaccine Group, including:
    a. Developed murine models and nonhuman primate (NHP) models for plague vaccine challenge protocols.
    b. Maintained stock cultures of the virulent challenge strains *Y. pestis*, C092 and C12, used in testing the protective efficacy of the F1-V fusion proteins (F1-V partial and F1-V whole).
    c. Cultured samples from *Y. pestis*-challenged animals to detect evidence of infection, e.g. bacteremia and thus to determine whether vaccination led to sterile immunity.
11. Since before 13 March 1996 to 27 August 1996 and thereafter, I prepared various *Y. pestis*,

C092 and C12, challenge preparations for use in experiments studying the efficacy of plague vaccines including F1-V fusion proteins, such as:

a. The long term efficacy studies conducted by Anderson. See Exhibit SW6.

b. Inocula of Y. pestis, C092 or C12, for aerosol challenge of mice vaccinated with either F1-V protein or a mixture of F1 + V or Plague USP vaccine on about 5 July 1996 as evidenced by pages 135 to 136 of Anderson's notebook #3739. See Exhibit SW7 (GA18).

c. Inocula of Y. pestis, C092 and C12, for aerosol challenge of mice vaccinated with either F1-V whole or a mixture of F1 + V on about 5 December 1996. See Exhibit SW8.

12. I have reviewed and analyzed the Titball patent and the three priority documents, UK 9505059, UK 9518946, and UK 9524825, and PCT/GB96/00571.

13. It is my opinion that prior to 13 March 1996, the filing date of PCT/GB96/00571, the inventors of the Titball patent had not conceived and/or reduced to practice a plague vaccine comprising purified F1 antigen fused to all or part of V antigen as nowhere do UK 9505059, UK 9518946, and UK 9524825 disclose isolating or purifying a protein comprising F1 antigen fused to all or part of V antigen from the host cell and other cellular components and/or administering a purified protein comprising F1 antigen fused to all or part of V antigen to a subject.

a. In fact, UK 9518946 is the first disclosure indicating a genetic vaccine or how a host organism may be transfected with DNA for F1 antigen and V antigen to result in a live vaccine, i.e. an attenuated host organism (such as Salmonella) which produces the antigen when administered to a subject.

b. As described in UK 9518946, the genetic vaccine or the live vaccine is administered to a subject such that the protein/antigen of interest is then produced in the subject.

c. UK 9518946 does not describe isolating the protein/antigen of interest from the host organism and purifying the protein/antigen of interest from other cellular components prior to administration to a subject.

d. The genetic vaccine or live vaccine described in UK 9518946 is not a purified protein comprising F1 antigen fused to all or part of V antigen which is isolated and purified from cells and other cellular components as claimed in the above-referenced application.

14. I have reviewed and analyzed the experiments and data of the Army Plague Vaccine Group and it is my opinion that the Army Plague Vaccine Group:

a. Conceived of a fusion protein comprising F1 antigen fused to part of V by at least **[redacted date which is before 13 March 1996]**.

b. Conceived of a fusion protein comprising F1 antigen fused to all of V by at least **[redacted date which is before 13 March 1996]**.

c. Conceived of and reduced to practice a purified fusion protein comprising F1 antigen fused to part of V by at least **[redacted date which is before 13 March 1996]**.

d. Conceived of and reduced to practice a purified fusion protein comprising F1 antigen fused to all of V by at least **[redacted date which is before 13 March 1996]**.

e. Conceived of and reduced to practice a vaccine against plague comprising a purified fusion protein comprising F1 antigen fused to part of V by at least **[redacted date which is before 13 March 1996]**.

f. Conceived of and reduced to practice a vaccine against plague comprising a purified fusion protein comprising F1 antigen fused to all of V by at least **[redacted date which is before 13 March 1996]**.

15. I declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Susan L. Welkos

Date: March 14, 2007

Turbo Gopher

MEETING NOTES FROM SUE WELK

MTG NOTES WELKOS

REDACTED

Art

Exhibit SW1

IBC Stuff

1) Generic protocol for Pesta
Jerry A.'s w/ R memo

2) Ward at Navy: Fl crystallization (with a protective Ab),
Marrow at Navy - expression vector for V.
will send him the V clone (Heath's)

3) Brubaker / Saratov group
b/ protein Vs. 100 LD50 of WT plague, SC, → done in Russia
~~today~~ using their V fusion.
Protein A
b) making a V fusion w/ enterokinase site

Kutyrev + Filippov - same as Leppla's (Stepanov) friends → not able to get them to come.

Protein A - (lcr)H

V from Y. pb was probably

Brubaker's hypothesis
V = colicin-like .... not involved in Ca++ regulation
H = immunity

4) J. Burns @ made ~ 8 MAb to Fl

will provide myeloma seed,
" " supernatants also.

Simpson's Fl clone (E.coli pKPR 1/5) : system to screen MAbs using opsonic activity → Tobery's style
Pretreat clone E.coli w/ Fl
Put on MΦ +/- MAb antibody cells
The E.coli Fl clone resists phagocytosis.
∴ see if the MAb allows phagocytosis

(b) Navy contract with P. Turnbull: To make stock cultures of diff. bact. + viral agents from around the world

5) Robert Ulrich
Naked DNA immunization: searching for an application.
LTR retroviral system

Use a rat cells
Provide him with Fl clone → (1) transfect directly ~~to~~ rat cells in vitro. Put cells sc into muscle.
(2) Inoculate animal directly with the DNA

Jerry A.
Preparative Super-Dex Column
Will exclude the stable nFl aggregates (2.5×10^6 MW) - get in void volume.
Other contaminants are retained.

Acetate dry → NaCl extract → 30% NH4SO4 cut
→ +/- 25% cut to remove pH6 Ag → Superdex/void vol.

Dave Heath
Cloned the ~~ler~~ VGH for GVH (2.1 kB) in pBLSkII (1) Sequenced the ends to prove was ok. (2) 2 internal V gene primers 168-275 aa au } see Brubaker's proposal

made fusion of Fl and V, using this internal sequence

W. Simpson's: Eco RC A Barn PCR done Fl caf Eco → pBL SkII

Dave's Fl construction
dropped the TAA at end of Fl gene.
Added 6nt for EcoRI site.
Added GC clamp
Made primers to get a Barn-Eco fragment on PCR

aa aa

(over)

Exhibit SW2

Gerry's colleague is looking for other regulators in Y. pestis (Katherine

Global thermoregulator

REDACTED

1. Dave's fusion of V-F1 need good monoclonal α V
   AFwill ask Brubaker.
   Also to get the monoclonal of Karu's Mab, from FDA

2. Abstract & Publication - group ~~endeavor~~ endeavor / authorship!

3. F1 purification + preps
   ① 32 mg of supernatant F1 - Andrew
   ② Dave's prep

   Cutter F1 looks diff. on gel than Gerry's extract

4. Mice to test our F1 preps:

   1) Parenteral challenge Mice w. 100 LD50's:
   - 4 diff. dose of the F1 preps, 2 doses of each prep
   - Cutter vaccine control: and F1 extracted from Cutter vaccine
   - Aluminum hydroxide adjuvant

   NEED
   1. F1 ELISA:
   Cindy Ross / D. Margitic
   Using F1 from W. Reed prep ?

   F1 capture ELISA ..... Applied Research says have developed one.
   Koch says can detect ≥ 5 ng F1/ml

   George Andrews:
   Chris Bolt setting up to do Equilibrium F1 ELISA
   Navy has an F1 fiberoptic detection system developed → F1 in serum detected.

5. One of the monkey's receiving 17 CFU had an IgM anti-F1 response. To check it's baseline serum for reacting to F1

6, EM - Gerry A. can do regular negative staining:
drop unfixed bacteria on slide / formvar-coated grids

↳ Status of bacterial pellets work / gold labelling?

7. Worsham's protocol to put mutator back into Y. pestis (IBC - 12/8)
sacB agrees = a new Bacillus agree --- must get full RAC review:

Anytime put DNA of foreign origin into Y. pestis (class 3) must get RAC approval.

Exhibit SW3

Col. T.'s Priorities [A] ① "a timeline algorithm"

② Get info from Cutter vaccine - as much as possible.

"Use Cutter in all appropriate protective scenarios"

"Augment with antibiotics."

Col T. being asked:

"Does ~~cipro~~ doxycycline & ciprofloxacin, etc. protect"

Why are we doing FI if British are also?

duplication

We need to meet with Brits to define collaboration

ex: "we need to press & be aggressive."

Definition of the plague ~~threat~~ threat & how are they en.

He wants a Brit to come here or one of us to go there to work in lab.

He wants the Brit. & U.S. scientists to get together.

REDACTED

In-House Plague Review

Exhibit SW4

① Anderson, George

F1 encapsulation vaccine exp. Kendo's carried 3"

(A) 1 shot immunization

48 days challenge (at d. 44)

DOX:

10 μg F1 in microcapsule + 10 μg F1 is "yes" (gave higher anti-F1 titers)

SC challenge vs. 67 LD50's

good protection

sterile immunity in spleens at 28 days post challenge

(B) Aerosol Challenge w/ increased dose

1 shot F1 alone - poor

" " encapsulated - not help

1 vs. 2 doses F1 + alhydrogel → much better w/ 2 doses

∴ no clear cut evidence that microencapsulation helps vs. aerosol challenge

2. Collaborative work w/ British (CBE)

Recombinant V Ag from British

∴ V Antigen is v. good vaccine

10 μg: days 0 + 30 → with Alhydrogel

challenge: day 58 Alhydrogel

092: ① 61 → 10^6 LD50s challenge SC ---- good survival

② Aerosol - good protection again! (at d 28!)

59-971 LD50s challenge

all sterile spleens 28 days post challenge

C12: ∴ excellent protection, SC challenge

aerosol challenge ... does not as high, not given as well as C92"

7/8 - low dose challenge: 84 LD50

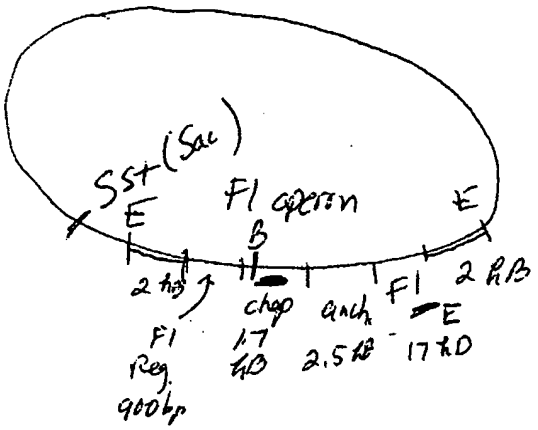
9/10 - high dose " : 193 LD50

Problem: Not low immunogen for AL titers

REDACTED Staff meeting : DAVE HEATH

Bubaher's - found immunogenic regions of V
= 168 - 275
fusion to a protein vector + deletion analysis

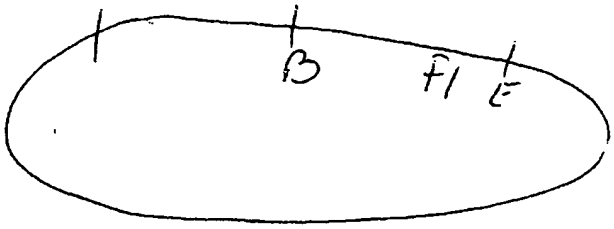
Dave fused this segment onto end of F1 gene



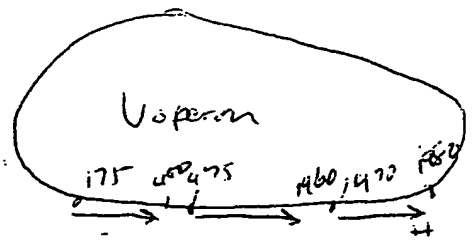
9.4 kB EcoRI fragment
need entire operon to get F1 expression (+ extend capsule made in E. Coli)

Bam-EcoRI subclone : get no F1 expressed.
Using a primer w/ EcoRI site,
fused EcoRI site next to last stop codon of F1 structure.
Took out Bam-Eco frag

∴ has entire operon w/ F1 with no stop codon!



reads 22 amino acids until runs into stop codon in F1



cc:Mail for: Dr. Susan Welkos

Subject: Re[2]: Mouse weights for aerosol run
From: LTC George Anderson 2/23/96 1:34 PM
To: Dr. Susan Welkos

Six months. These mice are part of a longterm challenge exp. Todays challenge is at 4 months postimmunization.

Subject: Re: Mouse weights for aerosol run

From: Dr. Susan Welkos

Date: 2/23/96 11:52 PM

George - Just for my info., how old are your mice at the time of these weights?

Thanks. Sue

Subject: Mouse weights for aerosol run
From: LTC George Anderson
Date: 2/23/96 11:39 AM

Louise, Use 29.98 gm as the average weight of the mice in my 2 runs (run 6 and 7) for the calculations of the challenge on 23Feb96. Range 24.9 - 34.3 gm.

plague-challenge.sc 2/1/96 2/1/96

P.I. = MAJ. G. Andrews and LTC George Anderson

Parenteral challenge of mice with the one challenge dose of **C092/C12**:

Plate counts: 2/5/96 -
USUAL CULTURE:
C12 - 10 x10e3/ml = **2 x10e3 cfu/dose** [conc. - 2.0 x 10e9/ml]
[**220 LD50s**]

PREACIDIFIED:
C12 - 13 x10e3/ml = **2.6 x10e3 cfu/dose** [conc. - 2.6 x 10e9/ml]
[**286 LD50s**]

Protocol: Long...

Exhibit SW7

Bleed: 21JUN96 Plate	Serum	Day: Day +204 Group	TREATMENT	26JUN96 F1 TITER	25JUN96 V TITER
1A	8906	GP13A	10F1+20V	10,240	20,480
1B	8907	GP13A	10F1+20V	1,280	40,960
2A	8908	GP13A	10F1+20V	10,240	81,920
2B	8909	GP13A	10F1+20V	10,240	81,920
3A	8910	GP13A	10F1+20V	5,120	20,480
3B	8911	GP13A	10F1+20V	20,480	20,480
4A	8912	GP13A	10F1+20V	10,240	40,960
4B	8913	GP13A	10F1+20V	40,960	10,240
5A	8914	GP13A	10F1+20V	2,560	81,920
5B	8915	GP13A	10F1+20V	10,240	81,920
6A	8916	GP13B	10F1+20V	5,120	20,480
6B	8917	GP13B	10F1+20V	20,480	81,920
7A	8918	GP13B	10F1+20V	2,560	40,960
7B	8919	GP13B	10F1+20V	20,480	163,840
8A	8920	GP13B	10F1+20V	10,240	81,920
8B	8921	GP14A	30ugF1-V	2,560	81,920
9A	8922	GP14A	30ugF1-V	2,560	81,920
9B	8923	GP14A	30ugF1-V	1,280	40,960
10A	8924	GP14A	30ugF1-V	1,280	40,960
10B	8925	GP14A	30ugF1-V	1,280	81,920
11A	8926	GP14A	30ugF1-V	2,560	81,920
11B	8927	GP14A	30ugF1-V	5,120	81,920
12A	8928	GP14A	30ugF1-V	1,280	40,960
12B	8929	GP14A	30ugF1-V	2,560	163,840
13A	8930	GP14A	30ugF1-V	5,120	81,920
14A	8931	GP14B	30ugF1-V	10,240	163,840
14B	8932	GP14B	30ugF1-V	20,480	81,920
15A	8933	GP14B	30ugF1-V	10,240	655,360
15B	8934	GP14B	30ugF1-V	5,120	81,920
16A	8935	GP14B	30ugF1-V	2,560	40,960
16B	8936	GP15	PlagueUSP	2,560	2,560
17A	8937	GP15	PlagueUSP	2,560	2,560
18A	8938	GP15	PlagueUSP	2,560	2,560
18B	8939	GP15	PlagueUSP	5,120	2,560
19A	8940	GP15	PlagueUSP	2,560	2,560
19B	8941	GP15	PlagueUSP	5,120	2,560
20A	8942	GP15	PlagueUSP	5,120	2,560
20B	8943	GP15	PlagueUSP	20,480	5,120
21A	8944	GP15	PlagueUSP	40,960	640
21B	8945	GP15	PlagueUSP	5,120	1,280
22A	8946	GP16	ALH alone	640	2,560
22B	8947	GP16	ALH alone	320	640
23A	8948	GP16	ALH alone	320	640
23B	8949	GP16	ALH alone	320	1,280
24A	8950	GP16	ALH alone	320	640
24B	8951	GP16	ALH alone	640	640
25A	8952	GP16	ALH alone	320	1,280
25B	8953	GP16	ALH alone	1,280	2,560
26A	8954	GP16	ALH alone	640	640
26B	8955	GP16	ALH alone	1,280	5,120
27A	F1/V	POOL		327,680	1,310,720
27B	Norm Mouse	POOL		320	2,560

V-titer too high, see control serum.

GEOMEAN:	Group	TREATMENT	F1 TITER	V TITER
	GP13A/B	10F1+20V	8,512	44,926
	GP14A/B	30ugF1-V	3,378	85,794
	GP15	PlagueUSP	5,487	2,229
	GP 16	ALH alone	520	1,194

For plague challenge of 5 Jul 96

140 LD50 C092
74 LD50 C12

data from Sue Welkos

RWL
8 Jul 96

plague-challenge.sc 7/5/96

7/5/96

P.I. = LTC George Anderson
40 mice, C092 - 100 LD50s
30 mice, C12 - 100 LD50s

Parenteral challenge of mice with C092/M.S. and C12/M.S.

1. Streak 1 slant each with the Master Seed of C092 and C12.
   Incubate 2 days at room temperature.
2. Harvest by suspending in 4-5 mls of HIB.
3. Read OD620 of a 1/10 dilution.
4. Adjust to OD 1.0

7/5/96:
Adjusted ODs and read final ODs on 1/2 dilutions:
Final OD = **1.064**, for C092
" " - **1.10**, for C12

C092/M.S.:

1. Prepare dose
5.0 -7.5x10e2/ml:
   (1) Add 0.2 ml OD 1.0 to 1.8 mls HIB.
   (2) Add 0.2 ml (1) to 1.8 mls HIB.
   (3) Add 0.5 ml of (2) to 4.5 mls HIB.
   (4) Add 0.5 ml of (3) to 4.5 mls HIB.
   (5) Add 0.5 ml of (4) to 4.5 mls HIB.
   (6) Add 4.0 ml of (5) to 36 mls HIB - - Pipet 10 mls into each of **3 tubes**:
   mice INOCULUM: 1 x 10e3/ml: ~200 cfu/dose

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2. Plating: The sample will be diluted in HIB and plated on SBAP:

suspension	Conc./ID	dilution	Total No. no. plates	plates
mice **Inoculum**	5x10e2/ml	undil, 10-1	**5** each	10

RESULTS:
7/5/96 doses: 1.4 x 10e3/ml, **280 cfu/dose** (**140 LD50s**)

7/12/96 doses: 6.5 x 10e2/ml, **130 cfu/dose** (**72 LD50s**)
7/18/96 doses: ____ x 10e2/ml, **cfu/dose** (**LD50s**) - canceled

C12/M.S.:

1. Adjust slant suspension to OD620 = 1.0.
Prepare dose
2.3 x10e3/ml:

(1) Add 0.2 ml OD 1.0 to 1.8 mls HIB.
(2) Add 0.2 ml (1) to 1.8 mls HIB.
(3) Add 0.5 ml of (2) to 4.5 mls HIB.
(4) Add 0.5 ml of (3) to 4.5 mls HIB.
(5) Add 1.0 ml of (4) to 9.0 mls HIB.
(6) Add 6.0 ml of (5) to 18 mls HIB (1/4) - -

INOCULUM, C12-100 sc LD_{50}s (910 cfu). Pipet 10 mls into each of **2 tubes:**
1 x 10e3/ml: ~200 cfu/dose

---

2. Plating: The Inoculum will be diluted in HIB and plated on SBAP:

suspension	Conc./ID	dilution	no. plates	plates
C12 **Inoculum**	2.3x10e3/ml	undil.	5	
		10-1	5	
		10-2	5	TOTAL-15

RESULTS:

7/5/96 doses: 3.36 x 10e3/ml, 6.7 x 10e2 cfu/dose (73.6 LD50s)
7/12/96 doses: 3.0 x 10e3/ml, 5.8 x 10e2 cfu/dose (63.7 LD50s)
7/18/96 doses: ____ x 10e3/ml, ____ x 10e2 cfu/dose (____ LD50s) – counted

Exhibit SW8

plague-challenge.sc12/5/96 Dec. 5, 1996

SUBCUTANEOUS CHALLENGES

P.I.s: MAJ J. Adamovicz, LTC Anderson, COL Friedlander
Experment: To decide whether F1-V or F1+V offers better protection against high dose parenteral challenges of C092 and C12.

No. mice	Strain	Dose [No. LD50s]	No. cfu/dose (0.2 ml)	No. cfu/ml inoculum
50	C092	10e2	1.8 x 10e2	9-10 x 10e2/ml
35	"	10e3	1.8 x 10e3	9-10 x 10e3/ml
35	"	10e4	1.8 x 10e4	9-10 x 10e4/ml
50	"	10e7	1.8 x 10e7	9-10 x 10e7/ml
50	"	10e8	1.8 x 10e8	9-10 x 10e8/ml
50	"	10e9	1.8 x 10e9	9-10 x 10e9/ml

No. mice	Strain	Dose [No. LD50s]		
70	C12	10e7	9.1 x 10e7	4.55 x 10e8/ml
50	"	10e8	9.1 x 10e8	4.55 x 10e9/ml
50	"	10e9	9.1 x 10e9	4.55 x 10e10/ml

C12:

Highest Dose (10e9 LD50s): NEED 4.55 x 10e10/ml - -
Need OD620 = 23 - 46 [based on an OD620 of 1.0 = 1-2 x 10e9/ml].
Volume needed = 25 mls

1. Streak a combination of 30 TBAB slants/plates with the Master Seed of C12.
   Incubate 3 days at room temperature.
2. Harvest by suspending each plate or slant in 4 mls of HIB.
   Should have a total of ≤120 mls cells.
3. Collect in a GSA bottle. Centrifuge for 8 min. at 8000 rpm.
4. Suspend the pellet completely in approx. 1/5 the original volume (ie., ~24 mls).
5. Dilute the concentrated suspension 1/10. Read OD620 of a 1/5 and 1/10 dilution of the diluted suspension, for final dillutions of 1/100 and 1/50:
   OD 1/100 - _____
   OD 1/50 - _____
6. Adjust the concentrated suspension to a final OD = **40.0** [35 - 45] in a volume of **24 mls** HIB.
   FINAL OD 1/100 - _____
   OD 1/50 - _____

7. Pipet 10-mls of the OD 40 suspension to each of 2 tubes.
   9.1 x 10^9 cfu/dose - **10e9 LD50 INOCULUM**
   [4.6 x 10^{10} cfu/ml inoculum]

8. Prepare the lower doses by diluting the OD 40 suspension: 10e8 and 10e7 LD50s.
   10e8: Add 2.5 ml OD 40 to 22.5 mls HIB.
   Transfer 10 mls of Tube (1) to each of 2 tubes, for the inoculum:

INOCULUM [10^8 LD50s] - [9.1x10^8/dose; 4.6 x 10^9/ml]

10e7: Add 2.5 ml of **10e8** tube to 22.5 mls HIB.
Transfer 10 mls of the dilution to each of 2 tubes, for the inoculum:
INOCULUM [10^7 LD50s] - [9.1x10^7/dose; 4.6 x 10^8/ml]

9. Dilute further and plate dilutions on SBAP:
(1) Add 0.2 ml of **10e7** Inoculum to 1.8 mls HIB
(2) Add 0.2 ml of (1) to 1.8 mls HIB.
(3) Add 0.2 ml of (2) to 1.8 mls HIB.
(4) Add 0.2 ml of (3) to 1.8 mls HIB.
(5) Add 0.2 ml of (4) to 1.8 mls HIB.
(6) Add 0.2 ml of (5) to 1.8 mls HIB.
(7) Add 0.2 ml of (6) to 1.8 mls HIB.

10. Plated on SBAP: 5(3), 6(3), 7(3)

12/9/96: RESULTS
-5 =
-6 =
-7 = _____ x 10e10/ml -

SUBCUTANEOUS

strain	target no. LD50s [no cfu]	no. cfu/dose	final no. LD50s
C12	10^9 [9.1 x 10^9 cfu]		

C092:

Highest Dose (10e9 LD50s): NEED 10 x 10e9ml - -
Need OD620 = 10 [based on an OD620 of 1.0 = 1-2 x 10e9/ml].
Volume needed = 25 mls

1. Streak 10 TBAB slants with the Master Seed of C092.
   Incubate 3 days at room temperature.
2. Harvest by suspending each slant in 4 mls of HIB.
   Should have a total of ≤40 mls cells.
3. Collect in a GSA bottle. Centrifuge for 8 ~~min. at 8000~~ rpm. yes
4. Suspend the pellet completely in approx 25 mls HIB.
5. Dilute the concentrated suspension 1/10. ~~Read OD620~~ of a 1/5 and 1/10 dilution of the diluted suspension, for final dillutions of 1/100 and ~~1/50~~:
   OD 1/100 - 0.115 → 11.5
   OD 1/50 - 0.202 → 10.1
   ok no adjustments needed
6. Adjust the concentrated suspension to a final OD = **10.0** in a volume of **24 mls**.
   FINAL OD 1/100 - 11.5
   OD 1/50 - 10.1
7. Pipet 10-mls of the OD 10 suspension to each of 2 tubes.
   2 x 10^9 cfu/dose - **10e9 LD50 INOCULUM** (0.2mls, sc)

[10 x 10^9cfu/ml inoculum]

8. Prepare the lower doses by diluting the OD 10 suspension:
   10e8 and 10e7 LD50s — $10^9, 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2$ LD_{50}s
   10e4, 10e3, and 10e2 LD50s

   10^8 LD50

   10e8: Add 2.5 ml OD 10 to 22.5 mls HIB.
   Transfer 10 mls of Tube (#) to each of 2 tubes, for the inoculum:
   INOCULUM [**10^8 LD50s**] - [2x10^8/dose; 1 x 10^9/ml]

   10e7: Add 2.5 ml of 10e8 tube to 22.5 mls HIB.
   Transfer 10 mls of the dilution to each of 2 tubes, for the inoculum:
   INOCULUM [**10^7 LD50s**] - [2x10^7/dose; 1 x 10^8/ml]

   10e6, 10e5, 10e4, 10e3, 10e2 ; as per Above.

9. Dilute further for lower LD50s:
(1) Add 0.2 ml of **10e7** Inoculum to 1.8 mls HIB [=10e6 LD50s]
(2) Add 0.5 ml of (1) to 4.5 mls HIB [=10e5 LD50s].
(3) **10e4**:
   Add 2.5 ml of tube (2) to 22.5 mls HIB.
   Transfer 10 mls of Tube (3) to each of 2 tubes, for the inoculum:
   INOCULUM [**10^4 LD50s**] - [2x10^4/dose; 1 x 10^5ml]
(4) **10e3**
   Add 2.5 ml of tube (3) to 22.5 mls HIB.
   Transfer 10 mls of Tube (4) to each of 2 tubes, for the inoculum:
   INOCULUM [**10^3 LD50s**] - [2x10^3/dose; 1 x 10^4ml]
(5) **10e2**
   Add 2.5 ml of tube (4) to 22.5 mls HIB.
   Transfer 10 mls of Tube (5) to each of 2 tubes, for the inoculum:
   INOCULUM [**10^2 LD50s**] - [2x10^2/dose; 1 x 10^3ml]

   10e2 LD_{50}s tube

10. Dilute further and plate dilutions on SBAP:
(a) Add 0.2 ml of (5) to 1.8 mls HIB.
(b) Add 0.2 ml of (6) to 1.8 mls HIB.

(b) 10^3 10^2 (a) (b)

11. Plated on SBAP: 4 (2), 5(3), 6(3), 7(3)

Counts: 10e9 LD50 Inoculum = $\frac{0.89 \text{ x } 10e10 \text{ cfu/ml}}{1.8 \text{ x } 10e9/\text{dose}}$ - - $\frac{}{1 \text{ x } 10e9 \text{ LD50s}}$

RESULTS:

strain	target no. LD50s [no cfu]	no. cfu/dose	final no. LD50s
C092	10e9 [2 x 10e9 cfu]	1.6 x 10^9	0.89 x 10^9
	10e8 [2 x 10e8 cfu]	" 10^8	" x 10^8
	10e7 [2 x 10e7 cfu]	" 10^7	" 10^7
	10e4 [2 x 10e4 cfu]	" 10^4	0.89 x 10^4
	10e3 [2 x 10e3 cfu]	" 10^3	" x 10^3
	10e2 [2 x 10e2 cfu]	" 10^2	" x 10^2

plague-sc challenge.12/5/96 Dec. 9, 1996

SUBCUTANEOUS CHALLENGES - Dose Calculations

P.I.s: MAJ J. Adamovicz, LTC Anderson, COL Friedlander

Experiment: To decide whether F1-V or F1+V offers better protection against high dose parenteral challenges of C092 and C12.

No. mice	Strain	Dose [No. LD50s]	No. cfu/dose (0.2 ml)	No. cfu/ml inoculum
50	C092	10e2	1.8 x 10e2	9-10 x 10e2/ml
35	"	10e3	1.8 x 10e3	9-10 x 10e3/ml
35	"	10e4	1.8 x 10e4	9-10 x 10e4/ml
50	"	10e7	1.8 x 10e7	9-10 x 10e7/ml
50	"	10e8	1.8 x 10e8	9-10 x 10e8/ml
50	"	10e9	1.8 x 10e9	9-10 x 10e9/ml

No. mice	Strain	Dose [No. LD50s]		
70	C12	10e7	9.1 x 10e7	4.55 x 10e8/ml
50	"	10e8	9.1 x 10e8	4.55 x 10e9/ml
50	"	10e9	9.1 x 10e9	4.55 x 10e10/ml

C092: 12/5/96 -

Counts: 10e9 LD50 Inoculum = $\frac{\textbf{0.80 x 10e10 cfu/ml}}{\text{1.6 x 10e9/dose}}$ - - 0.89 x 10e9 LD50s

RESULTS:

strain	target no. LD50s [no cfu]	no. cfu/dose	final no. LD50s
C092	10e9 [1.8-2 x 10e9 cfu]	1.6 x 10e9	0.89 x 10e9
	10e8 [1.8-2 x 10e8 cfu]	1.6 x 10e8	0.89 x 10e8
	10e7 [1.8-2 x 10e7 cfu]	1.6 x 10e7	0.89 x 10e7
	10e4 [1.8-2 x 10e4 cfu]	1.6 x 10e4	0.89 x 10e4
	10e3 [1.8-2 x 10e3 cfu]	1.6 x 10e3	0.89 x 10e3
	10e2 [1.8-2 x 10e2 cfu]	1.6 x 10e2	0.89 x 10e2

(C12) 1/23 challenge - 3 mice

plague-challenge.sc12/5/96 Dec. 5, 1996

SUBCUTANEOUS CHALLENGES

P.I.s: MAJ J. Adamovicz, LTC Anderson, COL Friedlander
Experment: To decide whether F1-V or F1+V offers better protection against high dose parenteral challenges of C092 and C12.

No. mice	Strain	Dose [No. LD50s]	No. cfu/dose (0.2 ml)	No. cfu/ml inoculum
50	C092	10e2	1.8 x 10e2	9-10 x 10e2/ml
35	"	10e3	1.8 x 10e3	9-10 x 10e3/ml
35	"	10e4	1.8 x 10e4	9-10 x 10e4/ml
50	"	10e7	1.8 x 10e7	9-10 x 10e7/ml
50	"	10e8	1.8 x 10e8	9-10 x 10e8/ml
50	"	10e9	1.8 x 10e9	9-10 x 10e9/ml

No. mice	Strain	Dose [No. LD50s]		
70	C12	10e7	9.1 x 10e7	4.55 x 10e8/ml
50	"	10e8	9.1 x 10e8	4.55 x 10e9/ml
50	"	10e9	9.1 x 10e9	4.55 x 10e10/ml

C12:

Highest Dose (10e9 LD50s): NEED 4.55 x 10e10/ml - -
Need OD620 = 23 - 46 [based on an OD620 of 1.0 = 1-2 x 10e9/ml].
Volume needed = 25 mls

1. Streak a combination of 30 TBAB slants/plates with the Master Seed of C12. Incubate 3 days at room temperature.
2. Harvest by suspending each plate or slant in 4 mls of HIB. Should have a total of ≤120 mls cells.
3. Collect in a GSA bottle. Centrifuge for 8 min. at 8000 rpm.
4. Suspend the pellet completely in approx. 1/5 the original volume (ie., ~24 mls).
5. Dilute the concentrated suspension 1/10. Read OD620 of a 1/5 and 1/10 dilution of the diluted suspension, for final dillutions of 1/100 and 1/50:
   OD 1/100 - _____
   OD 1/50 - _____
6. Adjust the concentrated suspension to a final OD = **40.0** [35 - 45] in a volume of **24 mls** HIB.
   FINAL OD 1/100 - 0.442 → 44.2
   OD 1/50 - 0.694 → 33.7 used not adjusted with HIB
7. Pipet 10-mls of the OD 40 suspension to each of 2 tubes.
   9.1 x 10^9 cfu/dose - **10e9 LD50 INOCULUM**
   [4.6 x 10^{10} cfu/ml inoculum]
8. Prepare the lower doses by diluting the OD 40 suspension: 10e8 and 10e7 LD50s.
   10e8: Add 2.5 ml OD 40 to 22.5 mls HIB.
   Transfer 10 mls of Tube (1) to each of 2 tubes, for the inoculum:

INOCULUM [10^8 LD50s] - [9.1x10^8/dose; 4.6 x 10^9/ml]

10e7: Add 2.5 ml of **10e8** tube to 22.5 mls HIB.
Transfer 10 mls of the dilution to each of 2 tubes, for the inoculum:
INOCULUM [10^7 LD50s] - [9.1x10^7/dose; 4.6 x 10^8/ml]

9. Dilute further and plate dilutions on SBAP:
(1) Add 0.2 ml of **10e7** Inoculum to 1.8 mls HIB
(2) Add 0.2 ml of (1) to 1.8 mls HIB.
(3) Add 0.2 ml of (2) to 1.8 mls HIB.
(4) Add 0.2 ml of (3) to 1.8 mls HIB.
(5) Add 0.2 ml of (4) to 1.8 mls HIB.
(6) Add 0.2 ml of (5) to 1.8 mls HIB.
(7) Add 0.2 ml of (6) to 1.8 mls HIB.

10. Plated on SBAP: 5(3), 6(3), 7(3)
1/27/97

~~12/9/96~~: RESULTS
-5 = TNTC
-6 = 43,43,45
-7 = 7,11,6

4.4×10^8/ml ÷ ~~x 10e10/ml~~ - 0.97×10^7/dose = $1.0 \times 10^{7,8,9}$ cfu/dose

SUBCUTANEOUS

strain	target no. LD50s [no cfu]	no. cfu/dose	final no. LD50s
C12	10^9 [9.1 x 10^9 cfu]		

C092:

Highest Dose (10e9 LD50s): NEED 10 x 10e9ml - -
Need OD620 = 10 [based on an OD620 of 1.0 = 1-2 x 10e9/ml].
Volume needed = 25 mls

1. Streak 10 TBAB slants with the Master Seed of C092.
Incubate 3 days at room temperature. 10-15
2. Harvest by suspending each slant in 4 mls of HIB.
Should have a total of ≤40 mls cells.
3. Collect in a GSA bottle. Centrifuge for 8 min. at 8000 rpm.
4. Suspend the pellet completely in approx. 25 mls HIB.
5. Dilute the concentrated suspension 1/10. Read OD620 of a 1/5 and 1/10 dilution of the diluted suspension, for final dillutions of 1/100 and 1/50:
OD 1/100 - _____
OD 1/50 - _____
6. Adjust the concentrated suspension to a final OD = **10.0** in a volume of **24 mls**.
FINAL OD 1/100 - 0.492 → 44.7
OD 1/50 - 0.164 → 33.1 used

7. Pipet 10-mls of the OD 10 suspension to each of 2 tubes.
2 x 10^9 cfu/dose - **10e9 LD50 INOCULUM** (0.2 mls, SC)

TO: Jeff Adamovicz, COL. Anderson, COL Friedlander

1/27/97: RESULTS: Plate counts: Sc Challenge Inocula

SUBCUTANEOUS

strain	target no. LD50s [no cfu]	no. cfu/dose	final no. LD50s
C12	$10^{7,8,9}$ [9.1 x 10^{9} cfu]	9.7 x 10e7,8,9	**1.0 x $10^{7,8,9}$**

Sue

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PREVIOUS POSITIONS: 1) 1980-1983, Assistant Professor
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2) 1983-1988, Investigator (GS-12)
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3) 1988- 1999 (GS-13)
4) 1999-2006, Investigator (GS-14, Microbiologist /403/ DB-III)
5) 2006 – present, Investigator (GS-15, Microbiologist /403/ DB-IV)

EDUCATION:

University of Delaware-Newark, DE B.A. June 1972 Biology

University of Florida, Dept. of Immun.
and Med. Micro., Gainesville, FL M.S. December 1973 Med. Microbiology

University of Florida, Gainesville, FL Ph.D. August 1977 Med. Microbiology

Uniformed Services University of
the Health Sciences-Bethesda, MD 1977 - 1980 postdoctoral

TITLE OF M.S. AND PH.D. THESIS:

M.S. - Identification of Salmonella with the Bacteriophage 0-1

Ph.D. - The Pathogenesis of Malabsorption in the Stasis Syndrome: The Role of Bacterial Overgrowth

CERTIFICATIONS/OTHER TRAINING:

1. National Registry of Microbiologists - Registered Microbiologist
   Pathogenic bacteriology and virology certification through 1994.
   Research and Development Microbiology since 1994
2. Techniques in Electron Microscopy - EVMS continuing education, 1982.
3. BASIC Programming - DP120 Frederick Community College, 1983.
4. Advanced Bacterial Genetics - Cold Spring Harbor, NY, 1984.
5. Methods in Oligonucleotide Site - Directed Mutagenesis, Catholic University, Washington, D.C., 1985
6. Medical and Experimental Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, 1989.
7. Polymerase Chain Reaction Techniques, Catholic University, Washington, D.C., 1991.
8. LEADS [Leadership, Education, and Development] course for supervisors, Frederick, MD, 13-17 September 1993.
9. Good Clinical Practices, Frederick, MD, 21-22 June 1994.
10. Microscopy/Photomicrography Workshop, American Type Culture Collection, Rockville, MD, 1 - 3 October 1997.
11. Two Hybrid Selection: Identification and Characterization of Protein-Protein Interactions, Foundation for Advanced Education in the Sciences, National Institutes of Health, Bethesda, MD, 25 - 27 February 1998.
12. Fundamentals of Systems Acquisition Management (ACQ101), Defense Acquisition University, completed 5 March 1999, CEU: 4.7
13. New Technologies Driving Microbiology into the 21st Century: Applying Genomics, Microarrays, and Combinatorial Chemistries to . . . Vaccine Development", Amer. Society for Microbiology, Nov. 1999, Philadelphia, PA.
14. Expression, Detection, and Purification of Recombinant Proteins in Prokaryotic and Eukaryotic cells, Foundation for Advanced Education in the Sciences, National Institutes of Health, Bethesda, MD, 11 - 13 November 2000.
15. Introduction to the FDA Good Laboratory Practices (GLP) Regulations, Frederick, MD, 30 April – 2 May 2002.
16. Proteomics, Principals and Methods, Foundation for Advanced Education in the Sciences, National Institutes of Health, Bethesda, MD, 4 - 8 October 2004.
17. Intermediate Medical Acquisitions Course (Army Medical Research and Materiel Command, MRMC). Provided 40 h towards continuing education requirement in defense acquistion and towards completion of ACQ201 of the Defense Acquisition University, Mt. Pleasant, MD, September 13 - 17, 2004.

18. Operator training course for FACsCalibur flow cytometer, BD Biosciences, Inc. Balerica, MA. July 25 – 29, 2006

HONORS AND SCHOLARSHIPS:

H. Rodney Sharp Academic Scholarship (1968 - 1972) - University of Delaware
Outstanding Senior in the College of Arts and Sciences (1972)
Phi Beta Kappa - 1972
American Association of University of Women Fellowship (1975-1976)
Sigma Xi - Old Dominion University affiliate, 1983
Exceptional Performance Awards – several; most recent 8-10-06
Special Act or Service Awards: September 1997, and others
Excellence in Federal Career Award: Outstanding Professional, Gold metal - May 1995, Baltimore Executive Board
Commander's Award for Civilian Service: September 28, 2005

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11. Welkos, S.L. Pathogenesis of Campylobacter enteritis - Animal model, p. 182, In: Monograph - The First International Workshop on Campylobacter Infections, Reading England, 1982.

12. Welkos, S.L. A modified broth-disk susceptibility test for Campylobacter. Eur. J. Clin. Microbiol. 1: 354-360, 1982.

13. Welkos, S.L. Experimental gastroenteritis in newly-hatched chicks infected with Campylobacter jejuni. J. Med. Microbiol., 18: 233-248, 1984.

14. Leppla, S., Robertson, D., Welkos, S., Smith, L., and Vodkin, M. Cloning and analysis of genes for anthrax toxin component. 1986.

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74. Bashaw, J., Norris, S., Trevino, S., Adamovicz, J., S. Welkos. In vitro correlate assays of immunity to infection with *Yersinia pestis*. Amer. Soc. for Microbiol. Gen. Meeting (106th), Orlando, Florida, May 25 – 29, 2006

75. Cote, C.K., Bozue, J. A., Moody, K. L., Welkos. S.L. Analysis of a novel spore-associated antigen in *Bacillus anthracis*. Amer. Soc. for Microbiol. Gen. Meeting (106th), Orlando, Florida, May 25 – 29, 2006.

76. Sanz, P., Brahmbhatt, T., Darnell, S., Cybulski, R., Bull, R., Cote, C., Welkos, S., and O'Brien, A. D. Exosporium proteins of Bacillus anthracis. Natl.Reg.Cent.Excell. Mtg, New York City, March 26-29, 2006.

77. Dimezzo, T. Ruthel, G. and Welkos.S. . *In vitro* Intracellular Trafficking of Virulence (V) Antigen during Infection by *Yersinia pestis*. 9th International Symposium on Yersinia, Lexington, KY, October 10-14, 2006.

78. Bashaw, J., Norris, S., Weeks, S., Trevino, S., Adamovicz, J., S. Welkos. Development of in vitro correlate assays of immunity to infection with *Yersinia pestis*. 25th Army Science Conference, Orlando, Florida, November 27 – 30, 2006.

79. J. Bozue, J., Cote, C., Moody, K., and Welkos, S. Fully virulent *Bacillus anthracis* does not require the immunodominant protein, BclA, for pathogenesis. Abst. #115.ASM Biodefense Research meeting, Washington, DC, March 2007.

80. Cote, C. K. et al. 2007 - [SoaA]. Internat.Conf. *Bacillus anthracis anthracis, Bacillus cereus* and *Bacillus thuringiensis*: *Bacillus* ACT 2007. Oslo, Norway, June 17-21, 2007.

81. Cote, C., S.L., Dimezzo, D. Banks, A.,Bradley, K., Welkos, S.L. Early interactions between *Bacillus anthracis* and macrophages that influence the balance between spore clearance and development of a lethal infection. Internat.Conf. *Bacillus anthracis, Bacillus cereus* and *Bacillus thuringiensis*: *Bacillus* ACT 2007. Oslo, Norway, June 17-21, 2007.

INVITED SPEAKER/Professional Activities:

1. Invited lecturer, *Bacillus anthracis* and research at USAMRIID. James Madison University Center for Integrated Science and Technology, April 18, 2006.
2. Scientific Steering Committee member and session Chairman, 6th International Anthrax Conference. Santa Fe, New Mexico, 25 - 29 September, 2005.
3. Contributor and Reviewer for W.H.O document, Anthrax in Humans and Animals: W.H.O. Guidance, 2005.
4. Invited speaker in NIAID/FDA/NIH/DHHS-cosponsored conference on Animal Models and Correlates of Protection for Plague Vaccines. NIH- Gaithersburg,MD, 13-14 October, 2004.
5. Doctoral dissertation committee member: T. Brahmbhatt, USUHS, Dept. Microbiology and Immunology, 2003 – present.
6. NIH/NIAID/FDA/DOD research study section reviewers: NIH/NIAID Biodefense FY2003-5 Program; NIH SBIR program 2003, Army SBIR 2003
7. Invited participant on NIAID panel of 18 March 2003: Expert Consultation on Monoclonal Antibodies for anthrax rPA
8. Invited speaker in NIAID conference on Tularemia and Plague Vaccine Developments. NIH-Bethesda, MD, 21-22 Nov. 2002.
9. Scientific Committee member and session cochairman, 5th International Anthrax Conference. Nice, France, 30 March - 4 April, 2003.
10. Lecturer at Uniformed Services University of the Health Sciences, Microbial Pathogenesis graduate course - Anthrax. 1983 - 2001.
11. Student seminar speaker: Anthrax. USUHS, November 2001
12. Invited speaker in the Frederick Faculty Seminar Series: "Immunity to Anthrax: The Potential Role of Antibodies Against the Toxins in Protection Against the Initial Spore Infection", Ft. Detrick. May 5, 1999.
13. Invited speaker at International Conferences on Anthrax: Winchester, UK, 1989 and Plymouth, UK, 1998.
14. Moderator of ASM scientific session on bacterial pathogens, 1993

Patent/patent applications

1. Lee, J. et al. Anthrax Vaccine. Patent No. US 6,770,479 B1, issued August 3, 2004.

2. Ivins, B. et al. Method of making a vaccine for anthrax. US 6,387,665 B1, May 14, 2002
3. Recombinant F1-V Plague vaccine. RIID 9608; 08/899,716 . Issued 2003.